DEVELOPMENT OF MICROBIAL AGENTS FOR THE CONTROL OF ACRIDIDAE

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ABSTRACT

The pathogenic microorganisms that are considered potentially useful as applied control agents include Entomopox viruses and the Protozoa, particularly *Malameba locustae, Nosema locustae, Nosema acridophagus,* and *Nosema cuneatum.* The selection and development of organisms as controlling agents depends on the host-environment-pathogen interrelationships that exist or will occur in the treated area. The factors concerning the pathogen that are considered important include virulence, host range, ease of mass production and prolonged storage, adaptability to efficient low cost application techniques, persistence, and potencial for registration as a microbial insectide. Field testing has demonstrated the feasibility of using *N. locustae* as a microbial agent, particularly for long-term control of grasshoppers (Orthoptera: Acrididae). Other organisms such as the viruses and *N. acridophagus* show potential as short-term controlling agents.

INTRODUCTION

A number of efforts were made toward the end of the nineteenth century to use microorganisms to control noxious Acrididae. Among the earliest reports of such studies were those dealing with the effects of various fungi on locusts by Kunckel D'Herculais and Langlois (1891) and Brongniart (1891). A few years later Edington (1898), in South Africa, and Brunner (1901), in the United States, reported natural epizootics among Acrididae caused by the fungus *Entomophthora (=Empusa) grylli.* During this same period, Kunckel D'Herculais (1900) reported on the natural and artificial propagation of fungi in Argentina. The efforts, reported in a review by Charles (1966), continued well into the present century. However, the most interesting and possibly the most controversial attempt to achieve microbial control of Acrididae began with a report by D'Herelle (1911) of an epizootic among Acrididae in Mexico caused by the bacterium then called *Coccobacillus acridiorum.* Later, D'Herelle (1914a,b) reported positive results when he used the bacterium to kill locusts and grasshoppers in parts of South America, particularly Argentina, and in Africa. However, subsequently other workers in Africa,

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particularly Sergent and L'Heritier (1914), Béguet (1915, 1916), and Sergent (1916), had highly variable results and in some cases lack of effectiveness. The work on *Coccobaccilus acridiorum* was then virtually discontinued, but investigations of other bacteria, and of their potential applied use, have continued.

It now is apparent that the efforts to use bacteria and fungi for control of Acrididae failed because these organisms generally require specific climatic conditions, namely high humidity, for growth and development. These organisms do play a significant role in regulating the densities of Acrididae in the tropical and subtropical parts of the world; however, they are not sufficiently dependable in the arid and semiarid regions of the world where grasshoppers and locusts are pests because of this dependence on high humidity. Therefore, microorganisms that are developed for applied use must be infective under a variety of conditions.

The Acrididae also are hosts to a number of pathogenic Protozoa and viruses that do not require specific temperature and moisture conditions for infection and that appear to offer great potential for applied use. These viruses include at least two Entomopox viruses and a picornavirus that we have isolated from grasshoppers in the United States, plus a picornavirus that was isolated in Australia from species of Gryllidae, but which also is pathogenic in Acrididae. One Entomopox virus, the grasshopper inclusion body virus (GIBV), has been fairly well characterized: the virions are quite large, about 350 nm long, contain DNA, and are occuluded in a protein inclusion body that apparently provides some protection to the virions (Henry *et al.* 1969). Viral replication occurs in the fat body of the host, and, when the virus is inoculated into third-instar nymphs, it usually causes death within 20 days. The host range includes species of the subfamilies Melanoplinae and Cyrtacanthacridinae. The other Entomopox is the *Arphia* pox virus (APV), which resembles GIBV both in structure and pathogenicity, but differs in that infections are limited to species in the subfamily Oedipodinae. The grasshopper picornavirus, known as the crystalline-array virus (CAV), is a small spherical virus, about 18 nm in diameter, that contains RNA, replicates in neuromuscular and muscular tissues, and causes death within 10 days when inoculated per os and within 6 days when injected (Jutila *et al.* 1970, Henry and Oma 1973). The host range includes species in the subfamilies Cyrtacanthacridinae, Melanoplinae, Oedipodinae, and probably the Gomphocerinae. When the cricket picornavirus was isolated by Reinganum *et al.* (1970), it could be compared to CAV and was then found to be structurally and pathogenically distinct. Within the Acrididae, we have inoculated it into *Schistocerca americana* (Drury) and *Melanoplus sanguinipes* (F.), but CAV, like all the pathogens discussed in this paper, undoubtedly has a much broader host range than currently documented.

The Protozoa that appear potentially useful include *Malameba locustae* (King and Taylor), an undescribed neogregarine, and 3 species of Microsporida. Also, a number of pathogenic protozoa are known and appear potentially useful, but have not been described.

Malameba locustae, an amoebic-type organism, infects the epithelial tissue of the midgut, gastric caecae, and Malpighian tubules of grasshoppers. It causes an insidious disease that results in density reductions more through reduced fecundity than through overt mortality. The host range, reported by King and Taylor (1936) and Taylor and King (1937), to include a large number of Acrididae, has now extended to include several species of Gryllidae. Although *M. locustae* is a serious pest in grasshoppers and locusts in laboratory cultures, Henry (1968), Donaldson (1971), and Henry and Oma (1975) reported that it can be easily controlled with antibiotics. The life cycle terminates in the development of a thick-walled cyst that is the resting stage when the organism is out of the host.

The undescribed neogregarine infects the digestive tract and fat body of various species of *Melanoplus*. The organism develops through several merogonic cycles as well as a sporogonic cycle that ultimately produces a large number of resistant spores. It appears to be quite virulent and is capable of causing density reduction through increased mortality as well as reduced fecuntity. Although the host range is expected to be much wider than presently known, relatively little work has been done with this organism because of difficulty with experimental transmission.

The Protozoa that appear to offer the greatest applied potential are 3 species of Microsporida belonging to genus *Nosema*. *Nosema locustae* Canning was described by Canning (1953, 1962) from *Locusta migratoria migratorioides* (Reiche and Fairmaire) and *Schistocerca gregaria* (Forskal) in laboratory cultures in England. Henry (1969a), extended the host range to include many species of Acrididae as well as some Gryllidae and it now appears that this pathogen will infect most species of Acrididae. *Nosema locustae* infects the fat body of acridids and thereby deprives the host of its energy reserves. Although Henry and Oma (1974a) demostrated that it is the least virulent of the 3 species of *Nosema*, it is sufficiently virulent to cause increased mortality and reduced fecundity in applied field tests (Henry 1971a; Henry *et al.* 1973; and Henry and Oma 1974b).

Nosema acridophagus Henry, which was described by Henry (1967) from *Schistocerca americana* (Drury), infects various tissues of species primarily in the subfamilies Crytacanthacridinae and Melanoplinae. Henry (1969b) showed that it induced tumor-like growths on the digestive tract. These growths caused lesions through which bacteria entered the hemocoel, and death of the host resulted from septicemia. *Nosema acridophagus* is the most virulent of the Microsporida; death usually results within 10 to 15 days after inoculation (Henry and Oma 1974a).

Nosema cuneatum Henry, which was described from a species of *Melanoplus* (Henry 1971b), infects the adipose tissue, including the pericardium. The virulence exhibited by this species is intermediate to that expressed by the other species of *Nosema*. Infections result in increased mortality within 18 to 20 days after inoculation as well as in reduced fecundity among the survivors.

CRITERIA FOR SELECTION OF PATHOGENS

The pathogens discussed exhibit characteristics that indicate their potential value in applied microbial control of the Acrididae. However, decisions about where and how to use them depend on the host environment-pathogen interrelationships that exist in the area or that will be established following introduction of the pathogen. Each of the factors of this triad can be examined separately.

Important Considerations About the Host

Unlike most applied entomological endeavors in which research is directed at the control of a particular pest species, the endeavor to control Acrididae deals with a number of species. However, relatively few species compete with man or his livestock for preferred forage to the point that they would be considered economically important. For example, of the 200 or so species of Acrididae in western United States, only 12 to 15 are considered economically important; most species are not important because they do not consume valuable plants or do not increase to high densities. Indeed, as Mulkern *et al.* (1969) pointed out, some species might be considered beneficial because they feed totally or predominantly on undesirable plants. For obvious reasons, applied microbial programs should be directed to the control of densities of the economically important species, and the other species should be used to maintain the existing enemies and any introduced pathogen.

As demonstrated by Mulkern *et al.* (1969), some species of Acrididae exhibit wide ranges of preferred host plants whereas other species are limited to a narrow range of food plants. Thus, *Melanoplus sanguinipes* is an economically important omnivorous

species that consumes grasses, sedges, broadleaf plants, plant debris, and animal tissue; but *Amphitornus coloradus* (Thomas), also an economically important species, feeds predominantly on 2 or 3 species of grasses. In addition, the food habits of some species change during development; young nymphs of *M. sanguinipes* feed almost exclusively on organic debris and small broadleaf plants, but older nymphs show an increasing preference for grasses. *Amphitornus coloradus* feed on the same kinds of plants during their entire development. These food habits of grasshoppers not only are important in relation to economics, but are also important from the standpoint of efficient use of pathogens because the host must consume the infectious stages of protozoa and viruses.

Important Considerations About the Environment

Although grasshoppers and locusts inhabit most land areas of the world, those species of greatest concern occur in the semiarid regions. In these regions, the Acrididae exist in a delicate balance with their natural enemies, primarily pathogenic microorganisms, other insects, and insectivorous vertebrates. Furthermore, these regions are characterized by variable and changing climatic conditions that, among other things, disturb this balance and cause yearly fluctuations and irregular distributions in the densities of grasshoppers and locusts. The resulting pattern is one of periodicity of peak densities, which, in most areas, occur at about 7- to 15-year intervals.

The main reason grasshoppers and locusts are serious pests in the semiarid regions is because they compete with domestic livestock for forage, which is limited and which is generally used to the maximum by the grazing animals during some part of the year. However, the limited amount of available forage usually causes the per unit value of the land (hectare or acre) to be very low and, therefore, justification for control of grasshoppers and locusts must be based on the value of the forage that is to be protected. In western United States, densities in excess of 8 grasshoppers per yd^2 are considered economically important, though this economic threshold does not take into account the question of whether these grasshoppers are of economically important species. Generally one or two predominant species in any complex in an outbreak will account for 70-80 percent of all grasshoppers, and these are usually economically important. However, control programs usually are not initiated until the densities exceed about 15 grasshoppers per yd^2 and until there exists a visible threat to standing forage. Thus, there is a difference between the economic and control thresholds. In the final analysis, all the factor, including the existing natural enemies, periodic outbreacks, economic and control thresholds, etc., must be considered in selecting and developing microbial agents.

Important Characteristics of Pathogens

From the characteristics of the host and environment, one can predict the characteristics of pathogens that are important and then select the pathogens that will be most effective. The characteristics that I considerer most important for effective use against the Acrididae are virulence, host range, efficient mass production and prolonged storage, ease of application, persistence, and probability of registration.

Virulence.—Every pathogenic agent exhibits a certain ability to produce disease in a particular host, and pathogens of Acrididae can be rated relative to each other in their ability to cause mortality within certain species; for example, *N. locustae* is much less virulent than *N. acridophagus* when inoculated in *M. sanguinipes*. This relative virulence tends to be constant among all species of Acrididae, providing, of course, that the pathogen is capable of initiating the infection in the first place. In insects, unlike

vertebrates, acquired humoral responses to infectious agents are not active. Therefore, once a pathogen overcomes the mechanical barriers and reaches a suitable tissue, the insect will either be killed or debilitated to some degree by the disease.

Another basic fact is that the virulence, or disease-causing ability, is inversely proportional to the natural incidence of that pathogen. The least virulent of all pathogenic protozoa, the gregarines, organisms that inhabit the digestive tracts of the Acrididae and other insects, are very common and frequently occur at a 100 percent incidence within certain grasshopper populations. In contrast, the most virulent pathogen, the crystallinne-array virus, has never been isolated or observed in field-collected Acrididae. This situation may result from the interaction between these organisms over a long evolutionary period, an interaction that has insured the survival of both pathogen and host. The importance of this is that in developing a microorganism for applied use the ability of that organism to cause disease and ultimately kill grasshoppers can be predicted from the natural incidence.

The selection of a highly virulent or a less virulent pathogen for a particular situation depends on the objective of the control program. Should the situation require rapid reduction in density, as in an outbreak that threatens a valuable crop, then a highly virulent organism should be selected. However, if long-term management of the densities of grasshoppers and locusts is required, as on low value rangelands, then a less virulent agent might be more effective. The development of *N. locustae* is being emphasized because it is sufficiently virulent to cause some short-term mortality but it is also sufficiently nonvirulent so as to persist in the population long after application. For example, one billion spores on 1.5 lb (680 gm) flaky wheat bran per acre (0.405 hectare) applied when the predominant species are third instar nymphs will cause about 50 percent reduction in density within 4 weeks, which is about the ovipositional period (Henry 1971a, Henry *et al.* 1973). Beyond the 4 weeks, the pathogen continues causing deaths, but it also reduces fecundity.

In the United States, some initial reduction in density is normally necessary because landowners and operators do not begin control practices until densities are well above the economic threshold. The short-term mortality achieved with *N. locustae* is then sufficient to reduce the densities to near or below the economic threshold and certainly below the control threshold. However, consideration has been given to the possibility of using *N. locustae* as a management tool rather than as a control agent. Such an approach would require periodic applications, about every 3 to 4 years, to prevent the development of damaging densities. The risk is that a situation might develop in which densities are maintained at such low levels that some existing natural enemies might be excluded to the point that they are no longer effective in contributing to control. This result might be analogous to excessive use of chemical insecticides by which natural enemies are totally eliminated, either directly or indirectly.

Host Range.— The host range exhibited by a pathogen is important because, as mentioned earlier, control is directed at a number of different species. In any one area where damaging populations develop, there may be anywhere from 20 to 50 species, but only 2 or 3 are dominant and economically important. However, the economic species may belong to different subfamilies so the pathogen must be capable of infecting most species in order to infect just the important ones.

The host ranges of pathogens of Acrididae generally show an inverse relationship with virulence: organisms that exhibit the most extensive host ranges are least virulent. Thus, *N. locustae* would be most useful for long-term control, but CAV or *N. acridophagus* would be more effective for short-term control, providing one or more of the predominant species are susceptible to infection. However, as demostrated in field tests (Henry *et al.* 1973), infection of all species in a complex is not necessary to achieve reductions in overall densities. For example, when the densities of the more susceptible species are reduced directly from disease following application of a pathogen, this reduction causes increased predation and parasitism of other species by the existing natural enemies, many of which have very wide host preferences. In recent field tests with *N. locustae,* numerous infections were observerd in 2 dominant species of *Melanoplus* that caused about 70 percent reduction in densities at 4 weeks post application, but very few infections were observed among the other 2 dominant species, *Aulocara elliotti*

(Thomas) and *Ageneotettix deorum* (Scudder). Nevertheless, the densities of these latter 2 species were reduced by about 50 percent compared with untreated checks (Henry *et al.* 1973). In these field tests, it appeared that the percentage of parasitism by entomophagous insects among the survivors of applications of *N. locustae* increases significantly following density reductions, a result that is much less likely when chemical insecticides are used because the chemicals are also toxic to parasitic insects.

Mass Production and Storage.– Mass production of any pathogen that is to be used for control is necessaty because outbreaks of grasshoppers and locusts are extensive and the organism must be applied over the entire affected area. Indeed, from the standpoint of effiency in use of equipment and personnel the production system should be operative during the entire year. This requires that the pathogen be stored for prolonged periods.

At the present, the viruses and protozoa that are being considered for microbial control of insects must be produced *in vivo*. Efforts to produce these organisms *in vitro*, as in cell cultures, are now showing promising results. However, the present indications are that monumental improvements will be required in cell culture techniques before the systems will be as practical or efficient as the insect host. Thus, all protozoa and viruses that are considered potentially useful against the Acrididae must presently be produced in insects. Unreported studies of the efficiency of producing *N. locustae* in *Melanoplus bivittatus* (Say) for large scale field tests demonstrated that *N. locustae* can be produced efficiently in this way. Moreover, the cost is relatively low, about $0.10 per acre (ca. $0.24 per hectare) dosage, which is considerably less expensive than present costs of chemical insecticide.

Both *N. acridophagus* and *N. cuneatum* are considered potentially useful for applied work as noted earlier. However, production of quantities sufficient for field testing has been difficult, partly because these pathogens are quite virulent and, partly because relatively few spores are produced in infected grasshoppers (Henry and Oma 1974b). However, recent studies have shown that these pathogens are infective in the corn earworm, *Heliothis zea* (Boddie), following special inoculative procedures, and it may be possible to produce large quantities of these spores at very low costs. Furthermore, assays have shown that spores, which are the infective stages of these organisms, produced in *H. zea* are much more infective and virulent in grasshoppers than are spores produced in grasshoppers. This use of alternative non-host insects for production may be potentially useful with other pathogens.

As Henry and Oma (1974b) reported, there is a significant loss in spore viability during storage of *N. locustae*. When spores are stored in water at -10° C about 90 percent of the viability is lost within 3 years. Also, there appears to be a serious loss in spore viability within 10 to 14 days after application to wheat bran prior to field application. Intensive studies will be necessary to develop procedures for preserving the viability of espores during prolonged starage and during field application. Such improved methods of preserving spore viability would significantly increase the efficiency of these pathogens and would reduce the cost of production and application.

Ease of Application.–Development of efficient low cost methods of appliction is essential for successful use of microorganisms against Acrididae because of the low values of the semiarid lands where grasshoppers and locusts are major pests. The development of ultra low volume (ULV) application was a significant factor in decreasing the cost of applying chemical insecticides and, similarily, microbials must be formulated and applied in concentrated forms in order to reduce airplane and equipment costs. However, because most pathogens must be consumed by the target insects, edible carriers must be used. Accordingly, spores of *N. locustae* are first applied to flaky wheat bran which is then applied at a rate of 1.5 lb./acre. With such treatment, one-tenth the number of spores are used to achieve results comparable to those obtained by ULV spraying of spores (Henry *et al.* 1977). But, carriers such as wheat bran, which is a bulky material, do increase costs of application compared with ULV application because of the reduced coverage per load. However, unlike ULV applications of chemical insecticides, full coverage of *N. locustae* or other pathogens is not essential, and retreatment of missed areas is not required. Also, microbials formulated in dry carriers can be applied at temperatures and wind regimens that cannot prevail during ULV applications of liquid materials. Therefore, there is greater efficiency in use of the airplane and crews, which reduces the cost. In addition,

the swath widths for wheat bran applications are regulated to a great degree by altitude of the airplane and so the swaths can be much wider than those recommended for ULV sprays. This means that the airplane is capable of covering more ground surface per swath, thereby reducing the flying time. In sum, the reduced coverage per plane load resulting from the increased bulk of the wheat bran can be balanced by other factors so the costs of applying wheat bran with a pathogen will probably be very comparable with the costs of applying a liquid spray.

Persistence.—A pathogen must be capable of persisting in the population following application and must continue to function as a controlling factor until the densities of the host are so low that they no longer support the pathogen. At this low point, the hosts are dispersed to such a degree that contact is infrequent and thus spread of the pathogen is reduced. Such a level of density should be well below the economic threshold.

Based on our numerous field studies, it appears that the persistence of a pathogen usually varies inversely with virulence, and so less virulent pathogens usually persist at lower host densities than do the more virulent pathogens. *Nosema locustae* continues to function as a controlling factor until the densities of grasshoppers are depressed to about 1 per yd^2, and infections by eugregarines, which are even less virulent, have been observed at fairly high frequency when host densities are lower than 1 per yd^2. In contrast, the results of unreported field studies demonstrated that infections by the Entomopox virus (GIBV) decreased markedly when host densities were reduced to about 3 to 5 grasshoppers per yd^2. Thus the choice of pathogen will determine to a great extent the level to which the host density will be reduced.

Registration.—In the United States, all microbial control agents must be registered as pesticides prior to general use. The authority for registration rests with the Environmental Protection Agency (EPA). Many other countries also have governmental bodies that regulate pesticides, and these administrative groups undoubtedly will approve or disapprove the use of various microbial agents. Therefores, if a pathogen is to be used, it must exhibit characteristics that will allow it to be registered for general use.
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The protocols for registration in the United States and other countries have not been defined, but the main factors will be safety and efficacy. In other words, the microbial must be shown to be safe to use, and it must have a predictable effect.

There is a good deal of evidence that virtually all protozoan and viral pathogens that infect insects and also some bacterial and fungal pathogens are specific for insects. The main exceptions are arthropod-borne disease organisms such as malaria, dengue, various plant pathogens, etc. However, a pathogen that is being considered as a microbial control agent must undergo extensive testing to demonstrate non-infectivity for nontarget organisms. For example, mice, rats, guinea pigs, rabbits, fish, birds, and bees have been inoculated with *N. locustae* by a number of methods and have never shown infectivity, pathogenicity, or toxigenicity. Therefore, *N. locustae* is probably safe though some additional testing and constant screening may still be required. *Nosema acridophagus, N. cuneatum,* and *N. locustae* are likely to be similarly safe. Also, the Entomopox viruses are up to demostrate specificity for certain groups of insects, but the similarity of these viruses to the vertebrate pox viruses means that they must be tested even more extensively and thoroughly. Indeed, this similarity could prevent this use altogether because public acceptance may be lacking. The case of picornaviruses such as the crystalline–array virus of grasshoppers may be quite different. Although these viruses appear specific to insects, the small size of their genetic complement and the close similarity to such notorious mammalian viruses as poliovirus will probably prevent consideration or testing as microbial agents. It is because of these considerations that the pathogenic protozoa are receiving most of the emphasis in the development of microbial agents for use against Acrididae.

Efficacy of a product is important from the standpoint of consumer protection, and microbial insecticides, like chemical insecticides, will be labeled as to expected effectiveness. Effectiveness of chemical insecticides is determined by the toxicity per unit of active ingredient. This system, or one very similar to it, can be used with highly virulent organims that cause predictable mortality within a short period after application. However, the system will not be useful with less virulent organims that are being

developed for long–term control because the mortality and density reductions caused by these organims may not be obvious or predictable. The most appropriate alternative parameter for determining the efficacy of such pathogens may be the infectivity, that is, the percentage of infection that the formulation will produce in certain target species. This is the system used in defining the efficacy of formulations of *N. locustae* and it will probably be equally useful for most microbial agents developed for use against grasshoppers and locusts.

CONCLUSION

The development of any pathogenic microorganism for use against an insect is a very complex and involved process, and the development for use against grasshoppers may sometime be more difficult than for other agricultural pest such as the Lepidoptera. The major difficulties are: (1) the large number of different grasshopper species involved, each of which exhibits a particular susceptibility to each pathogen, (2) the highly irregular densities over both time and space and the irregular public support for control, (3) the fact that some important species are migratory, and (4) the fact that outbreaks are usually very extensive and may cover thousands of acres of relatively low–value land. As a result, microbial agents must be infective to a large array of species, persistent, effective under a variety of situations, and inexpensive.

The characteristics or factors that make grasshoppers and locusts highly suitable objects for microbial control include: (1) economic thresholds such that low densities can be tolerated, (2) exposure of the insects in nature during the entire growth and maturation, which makes it easier to reach them with microbial agents, (3) omnivorous consumption of food materials by pest species which allows a greater choice of carriers and (4) the fact that grasshoppers and locusts are natural hosts to a number of pathogenic microorganims that can be manipulated. The first and fourth characteristics. Acrididae are of particular importance because they allow flexibility and adaptability in the selection and use of microbial agents. Because the economic thresholds often allow the existence of low densities, longterm control programs with less virulent and probably less expensive organims can be employed in management programs. Microbial agents are now that will provide effective long–term control. Also, highly virulent agents are known that can give much the same quick control as chemical insecticides.

At present, no microbials have been developed to the point where they can be put into general use against the Acrididae. In fact, only *N. locustae* has been developed to the point where it has shown good potential for applied use. Other known pathogenes, particularly *N. acridophagus, N. cuneatum,* and *M. locustae,* have characteristics that would be as useful or even more useful than those of *N. locustae* for control of grasshopper and locusts. However, these are by no means the only pathogenic microorganims available. Some protozoa and viruses known in the Acrididae have not been isolated or studied. Also, I am sure serious surveys for pathogenic microorganisms in these insects in other parts of the world will produce useful microorganisms. The isolation, characterization, and development of these organims will require increased effort and cooperation between researchers throughout the world. Hopefully, such concerted effort will result in the eventual use of microbial agents in helping to maintain the densities of Acrididae at tolerable levels.

SUMMARY

Attemps during the early part of this century to control the dependence of these organisms on specific climatic conditions, namelay high himidity, for germination and survival. Accordingly, microbial control of Acrididae in the arid and semiarid regions requires selection and use of pathogens that function independently of any predisposing effects of climate. Protozoa and viruses have been

isolated that are pathogenic for grasshoppers and locusts under varied climatic conditions and that appear potentially useful as biological control agents. The viruses from grasshoppers include at least two Entomopox viruses and a picornavirus. Included among the Protozoa are the amoebic organism *Malameba locustae* an undescribed species of Neogregarinida, and 3 species of Microsporida, *Nosema locustae, N. acridophagus,* and *N. cuneatum.*

Selection of a pathogen for development as a microbial insecticide demands careful analysis of the host–environment–pathogen interrelationship that exists or will be established in the area of use. Factors considered important in relation to the host include the species complex, the food habits of the species, the seasonal developmental patterns, etc. Factors relating to the environment include the kinds and abundance of existing natural enemies, the value of the forage to be protected, and the damage thresholds involved. In evaluating pathogens, consederation must be given to characteristics such as virulence, host range, mass production and storage potentials, adaptability to efficient low cost application techniques, persistence in nature, and potential for registration as a microbial insecticide.

Nosema locustae has been tested extensively as a tool for managing the densities of Acrididae in western United States. These studies have demontrated that application of spores of *N. locustae* will cause reductions in densities through direc mortality and decreased fecundity. Other more virulent pathogens such as the viruses *N. acridophagus* and *N. cuneatum* appear more useful as short–term microbial insecticides.

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